Anomalous Results Obtained in the Determination of Available Lysine in Casein Using 2,4,6-Trinitrobenzenesulfonic Acid

Abstract

When acid precipitated caseins were evaluated for their available lysine using 2,4,6-trinitrobenzenesulfonic acid, amounts greater than the total lysine content were found. Detailed investigation of several casein fractions showed that the α_{s1} variants and β casein yielded available lysine amounts consistent with total lysine. However, available lysine of the κ-fraction was higher than its total lysine content. This is ascribed to hexosamines which are part of the structure of this molecular species. This finding indicates that caution should be observed in using 2,4,6-trinitrobenzenesulfonic acid for the determination of available lysine in foods containing significant amounts of glycoproteins.

Introduction

Gupta et al. (4) have demonstrated that processing conditions can substantially alter the available lysine in dry milk. In view of the nutritional importance of this essential amino acid, we occasionally concerned ourselves with its content in dried dairy products undergoing development in our laboratory. The recommended chemical method for determining available lysine—using 1-fluoro-2,4-dinitrobenzene (FDNB)—was tedious and time consuming; the new method—using 2,4,6-trinitrobenzenesulfonic acid (TNBS)—described by Kakade and Liener (5) was immediately tested. We describe briefly the results obtained when determining the available lysine in caseins with this reagent.

Experimental Procedure

Crude casein was prepared by adjusting the pH of fresh skimmilk held at 35C to 4.6 with N HCl. After 10 min the precipitate was filtered, washed three times with distilled water and lyophilized. "Nutritious" casein, a variant acid precipitated product, was also prepared according to Reyniers (6). β-Lactoglobulin was prepared by the method of Fox et al. (3). The α_{s1} A and C caseins, β-casein and κ-casein were obtained from the Milk Properties Laboratory, EURDD, Wyndmoor, Pennsylvania. N-acetyl neuraminic acid was purchased from Cal Bio Chem[1] and *d*-glucosamine-HCl, *d*-galactosamine-HCl, ϵ-TNP-l-lysine-HCl and TNBS were purchased from Nutritional Biochemicals Corporation.

"Available" lysine determinations were performed as described by Kakade and Liener (5). Total lysine determinations were made on the Beckman Model 120C amino acid analyzer by the method of Spackman et al. (7). Nitrogen was determined by the micro-Kjeldahl procedure (2). All samples were dried overnight at 75C under vacuum before use.

Results and Discussion

To follow changes in the available lysine content of milk during processing by Kakade and Liener's method, we noted that in some cases the "available" lysine either equalled or exceeded the total lysine content of the milk. Since Kakade and Liener had already demonstrated that their method could be used to determine accurately available lysine in the whey proteins of milk, we decided to seek the source of our discrepancy in the casein fraction.

Table 1 shows the results obtained in our analysis of casein prepared two different ways, pure α-casein genetic variants, β-casein, κ-casein and the primary amine containing carbohydrate

TABLE 1. Casein analyses.

	Total lysine	Available lysine by TNBS[a]
	g/100g sample	
β-Lactoglobulin	11.2	9.79
Acid precipitated casein	6.12	6.76
"Nutritious" casein	6.93	7.41
α_{s1}-AA Casein	8.45	7.61
α_{s1}-CC Casein	8.72	7.40
β-Casein	6.36	6.11
κ-Casein A+B	5.22	6.01
N-Acetyl neuraminic acid	.....	0
d + Glucosamine	.....	13.2
d-Galactosamine	.....	7.55

[a] Average of three analyses in triplicate.

[1] Mention of brand or firm names does not constitute an endorsement by the Department of Agriculture over others of a similar nature not mentioned.

moieties of κ-casein shown to be present by Alais (1). The α and β caseins were known to be carbohydrate-free (8), and the "available" lysine contents determined by Kakade and Liener's procedure are either equal to or less than the total lysine present. However, the available lysine for κ-casein is 15% more than the total lysine value and the whole casein values are 10 to 15% high.

We believe these high values for available lysine can be partially accounted for by the hexosamine in the casein. As shown in Table 1 *d*-glucosamine and *d*-galactosamine yield high apparent available lysine values in spite of losses during the strongly acidic hydrolysis employed in Kakade and Liener's procedure. N-Acetyl neuraminic acid, also known to be present in κ-casein, has no effect on the color development as it is completely destroyed by hydrolysis with the formation of a large amount of humin.

From our data, the quantity of hexosamine known to be present in κ-casein is insufficient to account completely for the high values of available lysine found in casein. However the casein micelle may well contain other uncharacterized glycoprotein fragments which would interfere with the TNBS determination of available lysine in casein and which, in conjunction with the hexosamine known to be present, would be responsible for the anomalous values found. Therefore, Kakade and Liener's method may be considered unsuitable for the accurate determination of available lysine in foods containing significant amounts of glycoproteins and caution should be exercised in its general application. Where no interfering materials are present it provides the researcher with an improved method for the determination of available lysine.

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